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16. ABSTRACT

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Exposure to copper at 9.3 µg/1 and higher resulted in a change in the structure and function of the test systems while 4.0 µg/1 did not cause an effect relative to controls. Systems at 9.3 µg/1 were structurally similar to controls but at reduced levels of primary production, dissolved organic carbon production, and macroalgal growth. Substantial structural changes occurred at ≥30 µg/1 as these systems increasingly shifted from autotrophic to heterotrophic systems. Little evidence of adaptation to the copper stress occurred during the test period.

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STRUCTURE AND FUNCTION OF COPPER-STRESSED AQUATIC MICROCOSMS

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Laboratory aquatic microcosms were established using natural pond sediment and the organisms contained therein. After a 30-day development time, microcosms were exposed on a flow-through basis to six concentrations of copper with six replicates of each concentration. Measures of energy fixation, material cycling, and biological structure were measured periodically over a 32-wk period.

Exposure to copper at 9.3 μg/l and higher resulted in a change in the structure and function of the test systems while 4.0 μg/l did not cause an effect relative to controls. Systems at 9.3 μg/l were structurally similar to controls but at reduced levels of primary production, dissolved organic carbon production, and macroalgal growth. Substantial structural changes occurred at ≥ 30 μg/l as these systems increasingly shifted from autotrophic to heterotrophic systems. Little evidence of adaptation to the copper stress occurred during the test period.

Key words: microcosms; copper; toxicity; organic carbon; energy fixation; material cycling; biological structure; resistance; adaptation; community

INTRODUCTION

Historically, the major toxicological tools available for predicting potential adverse biological impacts on aquatic systems are laboratory toxicity tests. Each species is usually treated individually with no interactions with other species. The resultant data are applied to natural systems in an attempt to protect existing or promote desirable aquatic communities. However, it has been postulated that due to interactions, communities and ecosystems as a whole may respond differently than individuals isolated from those communities (Odum, 1971). Thus, evaluations of toxicity should also be made at the ecosystem level incorporating measurements of energy fixation, material cycling, and biological structure. Energy fixation is primarily due to plant photosynthesis, material cycling includes respiration, decomposition, and other forms of mineral cycling while biological structure consists of the composition and nature of the biological community present.

The impact of a toxicant on an ecosystem is a function of the ability of the ecosystem to remain stable when stressed. Many theoretical discussions on

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ecosystem stability have been presented in the past (Lewontin, 1969; Holling, 1973; May, 1973; Webster et al., 1975). While different authors have provided various terms for the components of stability, resistance was the major attribute considered in this study. From a toxicological viewpoint, the question of ecosystem resistance involves quantifying the amount of stressor required to deflect an ecosystem outside its normal boundary conditions. In addition, the ability of the system to acclimate to the stressor was considered. A return to normal boundary conditions during the exposure period would indicate that the system adapted to the stress and became less sensitive with time. The amount of a stressor to which a system can adapt is also an important consideration.

The objectives of this research were to determine the effects of a toxic stress on energy fixation, material cycling and biological structure of an aquatic ecosystem. Microcosms exposed to various concentrations of copper were the test systems used in this study. The large aquatic toxicity data base for copper (U.S. EPA, 1980; Hedtke, 1982) allowed the selection of concentrations covering a range of potential effect levels. Microcosms are increasingly being used as surrogates for natural systems due to their increased replicability, lower resource requirements for investigations, and the lack of environmental damage as might occur in exposing a natural system.

MATERIALS AND METHODS

Toxicant

Copper was delivered to the test systems in the form of $CuSO_4 \cdot 5H_2O$ dissolved in water. Based on the available information it was decided to test at nominal concentrations of 0, 10, 30, 90, 270, and 810 μg total Cu/l. Samples for total and dissolved copper were collected, acidified, and analyzed by flameless techniques with a Perkin-Elmer 5000 atomic absorption spectrophotometer according to recommended Perkin-Elmer procedures. Dissolved copper was operationally defined as that passing through a 0.45 μm membrane filter.

Analyses of sediments for copper were conducted after 16 and 32 wk. A known amount of dried (105°C), homogenized sediment was digested in a 100 ml volumetric flask by adding 20 ml of 1:1 sulfuric and nitric acid and boiling the solution down to approximately 1 ml over a 3 h period. Samples were diluted to 100 ml with distilled water and analyzed as above.

Microcosms

Natural sediment from ponds not previously exposed to toxicants was collected from the grounds of the U.S. EPA Newtown Fish Toxicology Station, mixed, and randomly distributed to 38 microcosm test chambers (Fig. 1). Large rocks, living plant material and snails were removed from the sediment to the extent possible prior to distribution to the chambers. Sediment depth was approximately 5 cm with

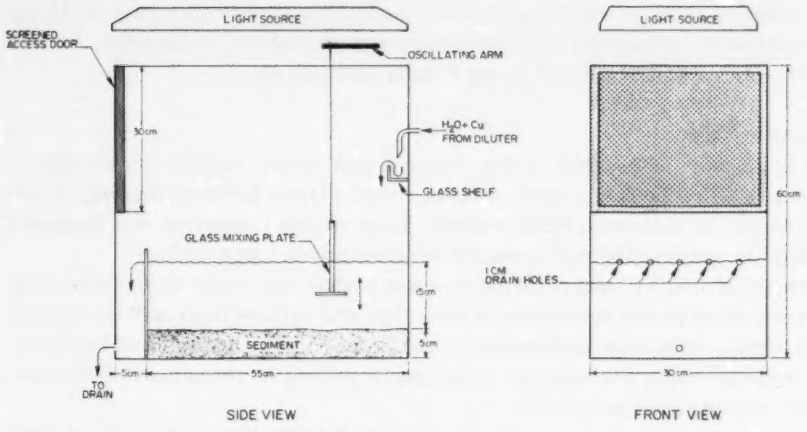


Fig. 1. Diagram of microcosm test chambers.

15 cm of overlaying water. One-hundred ml of water from an outdoor reservoir was delivered to each microcosm at 21 ± 1°C every 20 min resulting in a turnover time of approximately 3.5 days. Typical characteristics of the dilution water were as follows: alkalinity, 180 mg/l; as $CaCO_3$, hardness (EDTA), 200 mg/l as $CaCO_3$; total phosphorus, <0.2 mg/l; orthophosphorus, <0.1 mg/l; nitrate, 1.0 mg/l as N; ammonia, <0.1 mg/l as N; Na^+, 18 mg/l; Ca^{2+}, 52 mg/l; Mg^{2+}, 13 mg/l; Cl^-, 23 mg/l; and SO_4^{2-}, 23 mg/l. Light was supplied by Duro-Test Vita-Lite fluorescent bulbs on a 14 h on/10 h off daily photoperiod at 200 ft candles at the water surface. Each microcosm was entirely enclosed in glass to contain emerged animals with a removable screen door in the front for sampling. After 30 days one-third of the sediment was removed from each chamber, mixed together and randomly replaced to reduce the natural tendency of isolated systems to biologically diverge. At this time copper dosing began.

Thirty-six of the 38 microcosms were used in the copper exposure with the remaining two systems used to define initial system characteristics. Water and copper were delivered intermittently to six replicates at each concentration by a combination proportional and serial diluter at the same flow rate as previously indicated. Test chambers were arranged in a stratified random manner in six rows with each row consisting of one test system at each concentration. Systems were exposed at the appropriate copper concentration for 16 wk at which time three rows, making up three replicates at each concentration, were terminated and sampled as described later. The remaining three replicates at each concentration were exposed for an additional 16 wk at which time final sampling was conducted and the test terminated.

Energy fixation

Biweekly measurements of gross primary production in mg C/microcosm-day

were made on each test system using the diel pH–CO_2 method (Beyers et al. 1963). The method of calculation used the same basic procedures as described in Hall (1971). Results were converted to mg C/microcosm–2 wk.

Material cycling

Measurements of material cycling included total system respiration, changes in dissolved and particulate organic carbon, cattail (*Typha latifolia*) decomposition, and changes in sediment organic carbon. Total system respiration was measured biweekly in conjunction with measures of gross primary production.

Samples of dissolved and particulate organic carbon were taken daily, composited and analyzed as weekly composites of the inflow and outflow from each test system. Total organic carbon was measured with an Oceanographic International Total Carbon Analyzer using the ampoule technique according to recommended Oceanographic International procedures.

To determine the impact of copper on detritus decomposition, cattail leaves were added to each test system and decomposition rates determined. Previous to testing, dried leaves from the previous year's growth were collected from a local pond, gently washed with distilled water, cut into 2 cm sections and dried at 105°C for 4 h. Units of two sections were then randomly selected and weighed. These units were placed in small baskets made of perforated stainless steel with 1/4 inch holes. Each basket separately held six units of cattail sections and one basket was placed in each test system. Individual units were identified in the baskets so the comparisons with initial values could be made. The baskets were placed in the test systems during the seventh week of copper exposure. Samples for change in dry weight were collected after 3, 6, 9, 12, and 24 wk of exposure.

Sediment samples for total organic carbon were taken at week 0, 16, and 32 from test systems being terminated. After water was removed from a system, the sediment was thoroughly mixed and four 1.15 cm diameter circular core samples were collected, combined, and frozen for later analyses. Samples for analyses were dried at 105° for 4 h, weighed, and ground to a fine powder with a vibrating mill prior to TOC determinations. Ten replicate analyses were conducted for each sediment sample. The change in organic carbon in the sediments for a test system was calculated using the total surface area of the sediments, the surface area of the sample, the weight of the sample, the initial TOC of the sediments sampled at week 0, and the final TOC on a dry weight basis.

Biological structure

The biological components of the system were separated into the following categories: benthic macroinvertebrates, emerged insects, zooplankton, macroalgae, periphyton, and particulate organic carbon (see material cycling). Major sampling occurred at 0, 16, and 32 wk.

Benthic macroinvertebrates were sampled by removing the water from a test

system, thoroughly mixing the sediments, dividing the sediment into two equal parts, removing the sediment, and gently washing it through No. 50, U.S.A. Standard Testing Sieves (0.3 mm opening). One portion was used for taxonomic determinations and the other for biomass determinations. In addition to those organisms naturally present, five *Viviparus contectoides*, a pond snail, were added to each test system. Wet weights of the added snails were measured and the initial dry wt/wet wt relationship determined from additional sets of snails. Upon termination of a microcosm the dry weights of live *Viviparus* were measured.

Although snails were initially removed from the test systems, snails other than *Viviparus* were present in some test systems after 6 wk. These snails either developed from eggs or small snails in the sediment or from eggs that came in with the incoming water. When a test system was terminated, the snails found on the walls, on the algae, and in the sediments were removed for organism counts and dry weights. Initial values were assumed to be zero.

Oligochaetes were separated into three operational size classes: small, less than 2 cm crawling length; medium, greater than 2 cm crawling length and less than 2 mm diameter; and large, greater than 2 mm diameter. Due to the limited sample size, the small and medium oligochaetes were combined for dry weight determinations.

Each test system was checked daily for emerged insects and daily counts combined to give weekly totals. The numbers, sorted by gross taxonomic groups, were used as a measure of effect.

Zooplankton dry weights were determined at termination of a microcosm from water syphoned through a No. 200 (75 μm opening) stainless steel U.S.A. Standard Testing Seive. Visual determinations of relative abundance of ostracods, cladocerans, and copepods were also made.

Periphyton dry weights and chlorophyll *a* (Weber, 1973) were periodically determined from vertically oriented glass slides suspended at mid-depth in a Plexiglas slide rack.

Macroalgae dry weights were determined upon termination of a test system. Algae considered in this component did not include phytoplankton or periphyton. Algae attached to sediments were gently pulled from the soft sediment to include as much of the holdfast as possible.

Statistics

Data obtained from the various copper exposure systems were tested for statistical differences from control systems using analysis of variance (ANOVA) and Dunetts tests of significance (Steel and Torrie, 1960). A two-tailed test at 5% level of significance was used. When the standard deviations for an endpoint were proportional to the mean, a logarithmic transformation of the data was utilized.

TABLE I

Concentrations of copper in water during microcosm exposure

Nominal concentrations (μg Cu/l)	Input water (μg/l)	Total copper in water column (μg/l)	Percentage of input	Dissolved copper in water column (μg/l)	Percentage of total Cu
0 (control)	3.6 ± 1.2[a] (6)	1.4 ± 0.5 (40)	39	1.4 ± 0.6 (35)	100
10	15.5 ± 2.8 (6)	4.0 ± 0.7 (41)	26	3.6 ± 0.9 (34)	90
30	34 ± 6 (6)	9.3 ± 2.6 (49)	27	8.8 ± 2.2 (40)	95
90	110 ± 20 (6)	30 ± 7 (47)	27	25 ± 5 (34)	83
270	320 ± 50 (6)	90 ± 20 (36)	28	70 ± 20 (25)	78
810	820 ± 70 (6)	420 ± 50 (49)	51	360 ± 60 (34)	86

[a]Mean ± SD. Numbers in parentheses indicate number of samples analyzed.

RESULTS

Copper

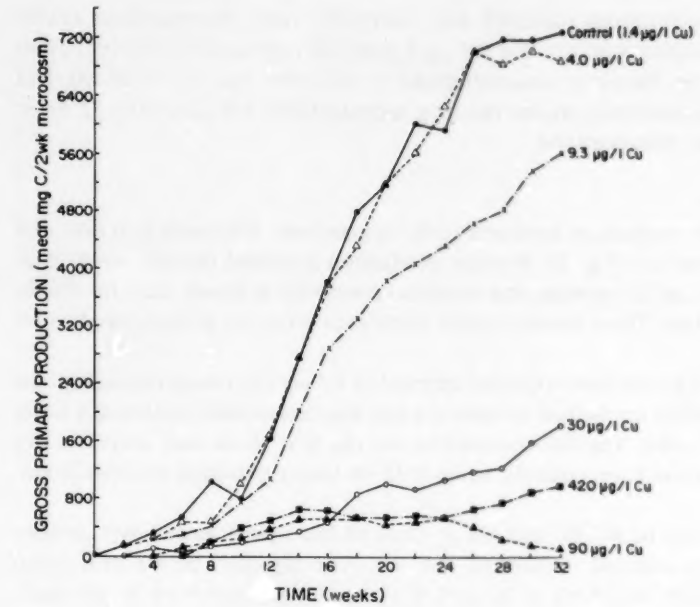
Concentrations of total copper measured in the water column of the test systems were 49–73% lower than the concentrations at the input to the systems (Table I). Therefore, mean measured water column concentrations were control (1.4), 4.0, 9.3, 30, 90, and 420 μg total Cu/l. These values will be used in the following discussions. Greater than 78% of the copper measured in the water column of the various systems was in the dissolved state. There was a slight increase in particulate copper in systems exposed to 30, 90, 420 μg Cu/l.

TABLE II

Concentrations of copper in sediments after 16 and 32 wk.

Exposure concentration (μg/l total Cu)	Sediment concentrations (μg/g dry wt)	
	16 wk	32 wk
Control - 1.4	33.7 ± 3.5[a]	32.0 ± 1.0
4.0	28.7 ± 1.2	33.7 ± 0.6
9.3	33.3 ± 1.5	37.3 ± 5.9
30	46.3 ± 9.1	56.7 ± 7.5
90	57.0 ± 7.8	113.7 ± 26.5
420	136.0 ± 28.2	145.7 ± 33.0

[a]Mean ± SD of 3 replicate systems.



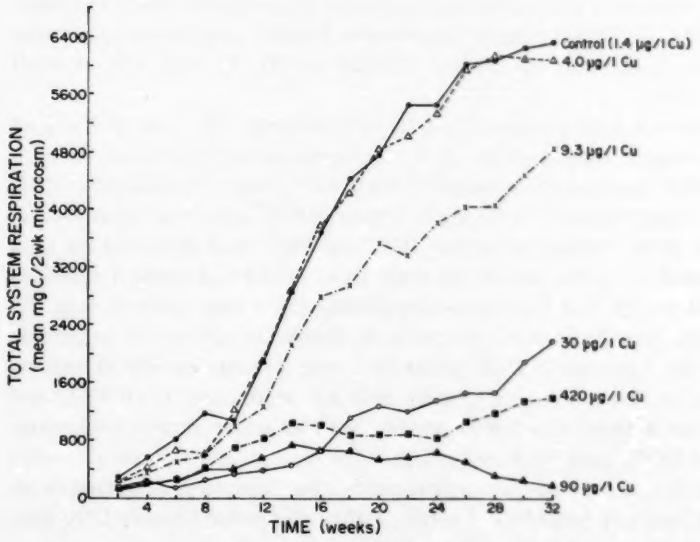


Fig. 2. Biweekly gross primary production and total system respiration of microcosms exposed to copper.

Sediment concentrations increased with increasing water concentrations (Table II). Sediments exposed to 4.0 and 9.3 μg/l were not appreciably different from background levels. However, concentrations in sediments exposed to 30, 90, and 420 μg/l were substantially greater reaching approximately 146 μg/g after 32 wk at the highest water concentration.

Energy fixation

Gross primary production measured in the systems varied according to time and copper concentrations (Fig. 2). Primary production increased through week 26 in controls and 4.0 μgCu/l systems and remained essentially at steady state for the remainder of the test. There was no overall significant effect on primary production at 4.0 μg Cu/l.

Mean primary production in systems exposed to 9.3 μg/l increased throughout the test period. Primary production in these systems was occasionally statistically lower than control systems. The total production for the first 16 wk was lower but not statistically different from controls, while at 32 wk total production was significantly lower.

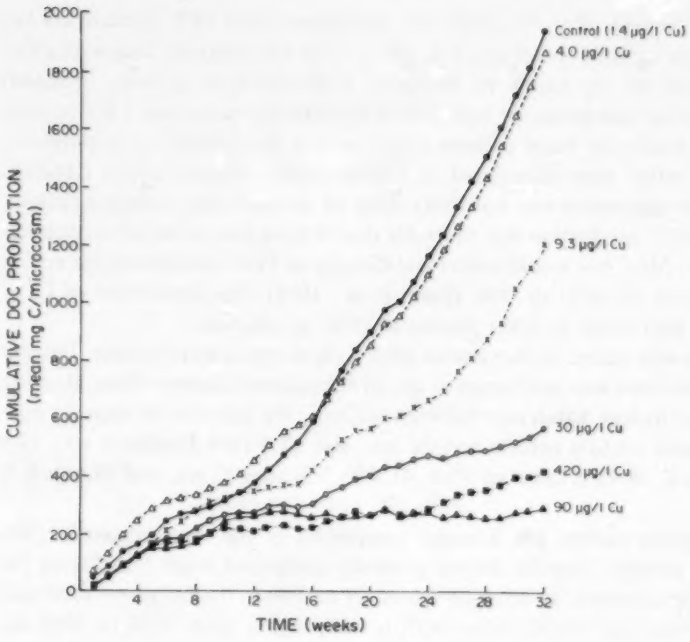
Systems exposed to 30, 90, and 420 μg Cu/l all had significantly lower primary production than controls throughout the test. The lag time before production became measurable was 4 wk at 30 μg/l, 6 wk at 90 μg/l, and 8 wk at 420 μg/l.

Material cycling

Total system respiration also varied during the test dependent on time and copper concentration (Fig. 2). With the exception of weeks 1 and 8, total system respiration was significantly lower only at systems exposed to 30, 90, and 420 μg Cu/l throughout the test.

The input water was from a common source and, therefore, the input of dissolved and particulate organic carbon (DOC, POC) was assumed to be the same for each system. Input DOC concentrations ranged from 0.7 to 1.8 mg C/l while input POC concentrations ranged from 0.1 to 0.3 mg C/l. Overall POC input was approximately 10% of DOC input. Values for output DOC and POC were measured for each system. From week 12 to the end of the study DOC production (output–input) in systems exposed to ≥ 9.3 μg Cu/l was substantially lower than controls (Fig. 3). DOC production must have been the result of metabolic activity by organisms within the systems. Increases in DOC production were strongly correlated with increases in algal biomass ($r = 0.952$), gross primary production ($r = 0.960$), and macroinvertebrate biomass ($r = 0.966$) but not with sediment carbon utilization. However, while DOC, algal biomass, and gross primary production continued to increase sharply during the study, macroinvertebrate biomass either decreased or remained stable between 16 and 32 wk. Therefore, the relationship between DOC production and copper was probably due to algal activity.

In contrast, POC production in systems exposed to ≥ 30 μg Cu/l was much



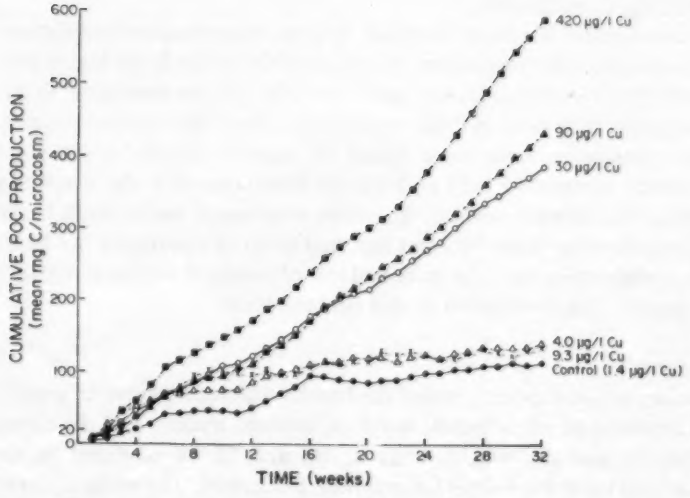


Fig. 3. DOC and POC production in microcosms exposed to copper.

greater than in controls (Fig. 3). There was no difference in POC production between the systems exposed to 9.3 μg Cu/l, 4.0 μg Cu/l and controls. Increased POC production could be the result of increased phytoplankton growth, increased suspension from the sediments, or increases in microbial populations. Limited data on chlorophyll within the water column suggested that phytoplankton populations were inhibited rather than stimulated at higher copper concentrations (Hedtke, 1982). Increased suspension was not likely since all systems were treated similarly. The increased POC production was probably due to increases in microbial populations or activity. Microbes would contribute directly to POC concentrations and in the transformation of DOC to POC (Paerl et al., 1975). The conversion of DOC to POC would also result in lower measured DOC production.

Cattail leaves were added to the systems after 7 wk of exposure to copper. Decomposition after this time was unaffected at any of the copper concentrations. Because there were no statistical differences between systems, the data for all systems were pooled. The mean (±SD) percent weight loss was 17.6 (±4.3) after 3 wk, 29.9 (±5.3) after 6 wk, 35.4 (±3.8) after 9 wk, 41.9 (±5.3) after 12 wk, and 49.4 (±6.5) after 24 wk.

Sediment organic carbon was a major component of the total organic carbon within the test systems. Organic matter generally comprised from 1 to 2% of the dry weight of the sediment. In all cases values for carbon in the sediments at 16 and 32 wk were lower than initial values with losses ranging from 1330 to 3060 mg C/microcosm. There were no differences between control values and any of the copper exposed systems at 16 wk. At 32 wk there was a significantly larger decrease in sediment TOC in systems exposed to 420 μg/l Cu (9800 mg C/microcosm) compared to controls (4600 mg C/microcosm).

Sediment carbon content is a result of inputs through sedimentation and outputs through aerobic and anaerobic respiration. The higher POC levels in the higher dosed systems should have resulted in greater inputs of carbon to the sediments. In addition, measured respiration rates in these systems were lower than control systems. However, these respiration rates were based on carbon dioxide production. Sediments in systems exposed to ≥30 μg/l Cu exhibited anaerobic characteristics immediately below the sediment surface. Anaerobic respiration would result in the formation of products other than CO_2 and reported levels of respiration are likely to underestimate total respiration. The increased loss of sediment carbon at 420 μg/l Cu suggests a greater underestimation at this concentration.

Biological structure

At the beginning of the exposure period the benthic macroinvertebrates present in the systems consisted of oligochaetes, mayflies, leeches, midges, and the added snails. The numbers and dry weights after 0, 16, and 32 wk of exposure are presented in Tables III and IV. Values for mayflies, planarians, and midges are not included in the tables. Mayflies were found only in the pre-exposure systems while

TABLE III

Numbers of organisms, by taxonomic groups, collected from laboratory microcosms exposed to copper

Measured exposure concentration (μg total Cu/l)	Exposure period (wk)	Number of organisms (no./system)					
		Oligochaeta			Gastropoda		Hirudinea
		Small	Medium	Large	Colonized snails	*Viviparus contectoides*	
Control - 1.4	0[a]	34 ± 17[b]	13 ± 7	9 ± 1	0	5	1.5 ± 0.7
	16	124 ± 58	34 ± 36	9 ± 3	259 ± 176	5 ± 0	4 ± 2
4.0	16	47 ± 21	41 ± 21	11 ± 3	191 ± 87	5 ± 0	27 ± 14
9.3	16	90 ± 75	16 ± 24	9 ± 4	191 ± 72	4 ± 1	13 ± 13
30	16	368 ± 75	127 ± 65	5 ± 1	0[c]	0.3 ± 1[c]	9 ± 16
90	16	630 ± 447[c]	86 ± 23	13 ± 6	0[c]	0[c]	45 ± 54
420	16	741 ± 59[c]	255 ± 191[c]	4 ± 4	0[c]	0[c]	1 ± 1
Control - 1.4	32	39 ± 45	31 ± 35	11 ± 3	205 ± 224	7 ± 2	14 ± 14
4.0	32	29 ± 19	14 ± 10	8 ± 2	173 ± 114	6 ± 2	10 ± 12
9.3	32	14 ± 12	5 ± 5	7 ± 3	227 ± 178	6 ± 1	23 ± 22
30	32	219 ± 156	82 ± 123	3 ± 6[c]	0[c]	0[c]	29 ± 30
90	32	518 ± 300[c]	109 ± 119	6 ± 5	0[c]	0[c]	2 ± 2
420	32	522 ± 151[c]	89 ± 50	0[c]	0[c]	0[c]	0

[a]Values obtained from two replicate systems sampled at the start of the exposure period.
[b]Values represent mean ± SD for replicate systems; 2 replicates at 0 wk, 3 replicates after 16 and 32 wk.
[c]Indicates statistical difference from controls, $P<0.05$.

TABLE IV

Dry weights for groups of organisms collected from laboratory microcosms exposed to various concentrations of copper

Measured exposure concentration (μg total Cu/l)	Exposure period (wk)	Dry weights (mg/system)						
		Oligochaeta		Gastropoda			Macroalgae	
		Small and medium	Large	Colonized snails	*Viviparus*[a] *contectoides*	Hirudinea	*Chara vulgaris*	*Vaucharia terrestris*
Control - 1.4	0[b]	14 ± 1[c]	350 ± 42	0	0	2 ± 1	0	0
	16	40 ± 30	679 ± 137	1315 ± 1131	1762 ± 411	42 ± 44	276 ± 189	2949 ± 545
4.0	16	57 ± 36	720 ± 139	328 ± 255	3544 ± 1909	45 ± 34	999 ± 846	2768 ± 1070
9.3	16	25 ± 35	781 ± 390	262 ± 52	2821 ± 1640	29 ± 26	516 ± 614	1672 ± 1690
30	16	313 ± 184[d]	405 ± 195	0[d]	397 ± 687[d]	21 ± 36	16 ± 27[d]	0[d]
90	16	122 ± 19	583 ± 218	0[d]	0[d]	46 ± 41	39 ± 36	62 ± 108
420	16	285 ± 192[d]	81 ± 79[d]	0[d]	0[d]	49 ± 84	1 ± 2[d]	0[d]
Control - 1.4	32	54 ± 67	716 ± 148	360 ± 214	2361 ± 282	32 ± 29	5686 ± 1297	14507 ± 2565
4.0	32	29 ± 20	606 ± 59	668 ± 844	2358 ± 383	20 ± 25	6292 ± 2481	12396 ± 4467
9.3	32	10 ± 6	433 ± 42	988 ± 741	2475 ± 202	49 ± 26	6663 ± 4688	4201 ± 5129[d]
30	32	217 ± 337	152 ± 263[d]	0[d]	0[d]	138 ± 184	678 ± 590[d]	0[d]
90	32	255 ± 208	377 ± 408	0[d]	0[d]	26 ± 26	5 ± 8[d]	0[d]
420	32	227 ± 101	0[d]	0[d]	0[d]	0[d]	0[d]	0[d]

[a]Values represent increase in dry weight over exposure period.

[b]Values obtained from two replicate systems sampled at the start of the exposure period.

[c]Mean ± SD for replicate systems; 2 replicates at week 0, 3 replicates at 16 and 32 wk.

[d]Indicates statistical difference from controls, $P < 0.05$.

only occasional planarians and midges were found in any of the sediment samples.

Oligochaetes and snails were the major macroinvertebrate components of the microcosms. Due to the uptake of copper by sediments, benthic macroinvertebrates were exposed to copper both through the water column and the sediments. Because it was not possible to distinguish between effects of the two exposure routes, reporting of effects is based only on the water concentrations. No effects on oligochaetes were found at 4.0 μg Cu/l. In general, exposure to 420 μg Cu/l resulted in increased small and medium oligochaetes and a decrease in large oligochaetes. Effects were sometimes seen at 30 μg Cu/l, while not at 90 μg Cu/l. The variability between replicates at these concentrations makes it difficult to reach firm conclusions about the impact of copper at these levels. However, increases in small and medium oligochaetes were found at levels of copper above 30 μg/l. Generalizations about large oligochaetes at copper levels below 420 μg/l cannot be made. The large oligochaetes were members of the Lumbricidae family. The small and medium oligochaetes consisted of the three genera of Naididae, *Uncinais, Paranais,* and *Pristina,* and the Tubificidae, *Tubifex.* There were no obvious toxicant dependent differences in the distribution of oligochaete taxa. The increases in smaller oligochaetes were apparently due to increases in existing populations rather than a shift to more tolerant populations.

Snails within the test systems were severely affected by exposure to copper. *Viviparus* added to the systems at the beginning of the exposure period for the most part survived exposures of ≤9.3 μg Cu/l (Table III). A single living *Viviparus* was found at 16 wk in one system exposed to 30 μg/l. No living snails were found in the remaining 30, 90, and 420 μg/l systems at 16 and 32 wk. No effects on *Viviparus* biomass (Table IV) were found with exposures to ≤9.3 μg/l.

Although all snails were removed from sediments prior to starting the exposure period, there were snails other than *Viviparus* in all the control systems and those exposed to 4.0 and 9.3 μg/l. These snails consisted of *Physa* sp. and *Helisoma campanulata.* None were found in the systems exposed to 30, 90, and 420 μg/l.

Midges were the only organisms that emerged from any of the test systems during the exposure period. Because of the variable pattern of emergence, weekly values did not give a consistent pattern in effects of copper. The cumulative total number of emerged midges gave more consistent indications of the effects of copper (Fig. 4). Effects were found at 420 μg/l throughout the test period. Effects were found at 90 μg/l by week 4 and 30 μg/l by week 5. In general, there was greater emergence during the second 16 wk period than the first 16 wk period.

Because zooplankton communities can undergo rapid changes in numbers and species composition, the samples at 16 and 32 wk can only be used to give a general impression of effects on zooplankton. Those systems exposed to ≤9.3 μg Cu/l were basically dominated by cladocerans with some ostracods. There were no appreciable differences in biomass. At the higher copper concentrations few cladocerans were seen and substantial reduction in biomass occurred. Ostracods were the only zooplankton visually present in the 420 μg Cu/l system.

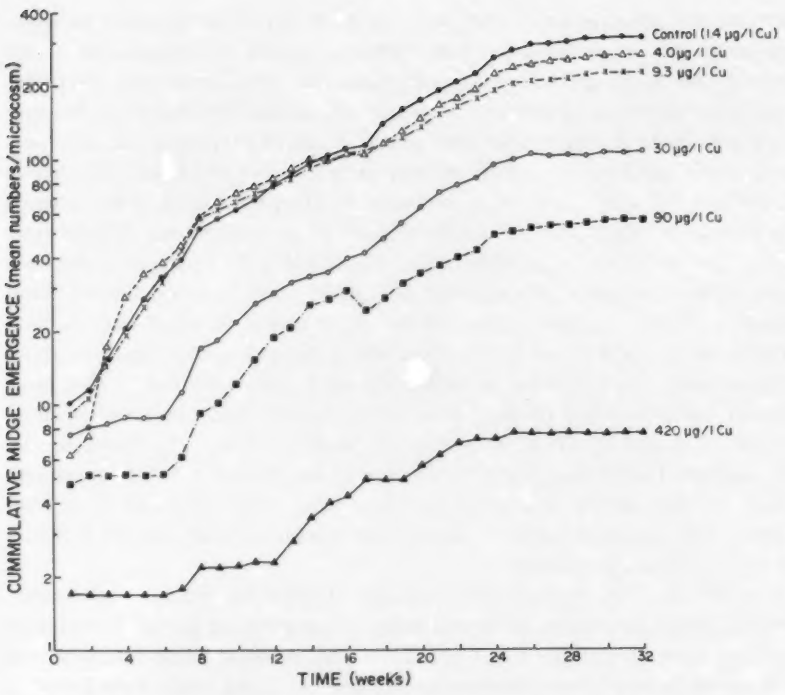


Fig. 4. Cumulative midge emergence from microcosms exposed to copper.

The growth of macroalgae was severely affected by exposure to copper at 30 µg/l and higher (Table IV). Macroalgae that developed in all systems were composed entirely of *Chara vulgaris* and a filamentous alga, *Vaucheria terrestris*. At the 16 wk sample there was a small amount of *Chara* present in two of the systems exposed to 90 µg/l while after 32 wk virtually no *Chara* was present. Although *Chara* was present in the 30 µg Cu/l systems at 32 wk, values for 30, 90, and 420 µg/l were all statistically different from controls. *Vaucheria* was more sensitive to copper than *Chara*. After 16 wk significant reductions in *Vaucheria* biomass were found at 30, 90, and 420 µg Cu/l. After 32 wk systems exposed to 9.3 µg/l also had statistically less of this alga than controls.

Periphyton measurements were variable and the effects of copper were not straightforward (Table V). An initial lag in growth occurred in systems exposed to 90 and 420 µg/l after which growth increased rapidly. The chlorophyll/dry weight relationship was, however, significantly lower in systems exposed to ≥ 30 µg/l suggesting that increases in biomass were due to bacteria and microinvertebrates rather than algae. Percent chlorophyll *a*, therefore, was a sensitive measurement of periphyton stress response.

TABLE V

Periphyton growth in systems exposed to copper

Exposure concentration (μg total Cu/l)	Exposure period (wk)					
	2	4	10	16	24	32
			Biomass/cm^2 (μg dry wt)			
Control	30[a] ± 10	80 ± 50	320 ± 100	390 ± 160	360 ± 50	420 ± 140
4.0	40 ± 10	70 ± 30	270 ± 70	300 ± 70	400 ± 60	460 ± 110
9.3	40 ± 10	70 ± 20	220 ± 90	270 ± 80	370 ± 160	360 ± 160
30	30 ± 5	15[b] ± 5	155 ± 100	260 ± 220	330 ± 160	790 ± 540
90	0[b]	15[b] ± 15	250 ± 90	540 ± 390	230 ± 210	70[b] ± 30
420	0[b]	0[b]	370 ± 140	500 ± 130	610[b] ± 130	960[b] ± 180
			Chlorophyll *a*/dry wt (%)			
Control		no data	1.2 ± 0.1	1.3 ± 0.3	1.3 ± 0.2	1.5 ± 0.2
4.0			1.4 ± 0.1	1.6 ± 0.2	1.2 ± 0.2	1.4 ± 0.3
9.3			1.8 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.6 ± 0.4
30			0.8 ± 1.1	0.5[b] ± 0.3	0.7[b] ± 0.2	0.4[b] ± 0.2
90			0.8 ± 0.2	0.5[b] ± 0.2	0.4[b] ± 0.3	0.6[b] ± 0.2
420			0.9 ± 0.4	0.7[b] ± 0.2	0.7[b] ± 0.3	0.7[b] ± 0.2

[a]Values represent mean ± SD for replicate systems; 6 replicates for weeks 0–16, 3 replicates for weeks 17–32.

[b]Indicates statistically different from controls, $P < 0.05$.

DISCUSSION

The exposure of the laboratory microcosms to copper resulted in effects on energy fixation, material cycling, and biological structure as a function of both exposure concentration and time (Table VI). After 16 wk effects were found at ≥ 30 μg/l while after 32 wk effects were found at ≥ 9.3 μg/l. Most effects were due to decreases relative to controls, however, POC outflow was greater than controls in the impacted systems. After 32 wk the components affected at 9.3 μg/l consisted of *Vaucheria*, DOC outflow and gross primary production.

At both 16 and 32 wk, the filamentous alga *Vaucheria* was one of the most sensitive components to copper. Decreased levels of this alga would also contribute to the observed decreases in gross primary production, total respiration, and dissolved organic carbon. This suggests that *Vaucheria* played a substantial role in the functioning of these aquatic systems. It also suggests that this alga could be a sensitive aquatic organism for future toxicological research.

Exposure to copper caused a shift in the structure and functioning of the laboratory ecosystems. Macroalgae were the major photosynthetic component and snails and oligochaetes were the major macroinvertebrate components in controls and systems exposed to 4.0 μg/l. Although qualitatively similar, systems at 9.3 μg/l had reduced rates of primary production and DOC production. In contrast,

TABLE VI

Significantly impacted components of microcosms exposed to various concentrations of copper

Exposure period (wk)	Copper concentration (μg/l)	Significantly impacted components
16	4.0	None
	9.3	None
	30	Snails, total macroinvertebrates, midge emergence, *Vaucheria*, POC outflow[a], gross primary production, total respiration, periphyton chlorophyll *a*/dry wt
	90	Same as 30
	420	Same as 30 plus large oligochaetes
32	4.0	None
	9.3	*Vaucheria*, DOC outflow, gross primary production
	30	Same as 9.3 plus snails, total macroinvertebrates, *Chara*, POC outflow[a], total respiration, midge emergence, periphyton chlorophyll *a*/dry wt
	90	Same as 30
	420	Same as 30 plus sediment TOC and large oligochaetes

[a]Significantly greater than controls; other effects listed were significantly lower than controls.

periphyton were the major photosynthetic component and oligochaetes were the major macroinvertebrate component in systems exposed to $\geq$ 30 μg/l. A larger proportion of the periphyton was microbes, POC production was greater and at 420 μg/l sediment carbon utilization was greater. Thus, at $\geq$ 30 μg/l the systems became more heterotrophic and microbially dominated.

Resistance to copper stress was defined by the concentration of copper which did not cause an effect on structure and function of the microcosms. After 16 wk the systems were capable of withstanding 9.3 μg/l Cu but not 30 μg/l while after 32 wk the systems could withstand 4.0 μg/l but not 9.3 μg/l. Exposure time, therefore, was an important factor in evaluating the resistance of the system. Either the systems became less resistant with time or the ability to detect the affect of copper increased with time.

The sensitivity of the measured responses was considered an indication of resistance. Equally important to the utilization of system-level responses in toxicity evaluations is whether systems are able to adapt to the perturbation. An increase in resistance, or apparent effect concentrations, over time was considered an indication of adaptation. In the affected systems there were increases in the magnitude of some system properties, e.g. gross primary production at 420 μg/l Cu. These increases, however, were not sufficient to change the evaluation of resistance. Thus, system components initially stressed by copper did not adapt and develop characteristics of the unstressed systems.

In order for ecosystems to adapt to a stressor at the system level, there must either

be adaptation by existing organisms or replacement by organisms that can perform the same function and are tolerant to the stressor. Replacement requires that the organisms already be present or must be introduced. While not specifically intended, the source of water for the test system supplied many types of organisms. In other tests conducted with the same water supply at the same laboratory, filamentous algae, snails, midges, and a variety of zooplankton have colonized otherwise clean aquaria. Undoubtedly phytoplankton and microbes also entered the systems through the water supply. Of the major components of the system which were identified, only the oligochaetes may not have had replacement capability. Overall, oligochaetes were not adversely affected by copper exposures. Therefore, it is felt that adequate opportunity for tolerant species to colonize the microcosms was provided.

The determination of adaptation depends in part on the time frame within which responses are measured. The degradation of cattail sections was one response where no effect of copper was observed. The cattails were added after the microcosms had been exposed to copper for 7 wk. The first measurement of degradation was after the cattails had been exposed for 3 wk. Based on this time frame, cattail degradation was resistant to copper. However, Jonas (1981) found that the microbial degradation of holocellulose was reduced by copper only during the first 12 days. After that time, degradation rates in the systems exposed to copper actually exceeded that of control systems. The microbial populations within the systems studied by Jonas were able to adapt to copper. In the current study an initial short-term effect of copper on cattail degraders would not have been observed due to sampling schedule. Therefore, it is not possible to conclude whether the microbes were actually resistant or adapted to copper.

The results of this study cannot be directly compared with those from traditional single-species tests. The system-level measures of effect utilized cannot be obtained in single-species tests while data on the toxicity of copper to the individual species found in the microcosms are not available. However, these results can be compared with recent national water quality criteria for copper which are based on single-species laboratory tests. The 1980 criterion (U.S. Environmental Protection Agency, 1980) recommended a 24-h average concentration not to exceed 5.6 μg/l while the latest version of this criterion (U.S. Environmental Protection Agency, 1984) assumes a hardness-related long-term concentration. In waters of hardness equal to that used in this study (200 mg/l as $CaCO_3$) the recommended 30-day average concentration is 20 μg/l. Effects in the microcosms were found at 9.3 μg/l but not at 4.0 μg/l, supporting the 5.6 μg/l recommendation.

In summary, copper affected nearly all aspects of both the structure and function of laboratory aquatic microcosms. The degree of impact was a function of both exposure concentration and time. After 32 wk systems were still resistant to exposures of 4.0 μg/l total Cu. Above this level there was an increasing shift from autotrophy to heterotrophy with increasing copper concentration. In general, adverse effects

were detected at lower concentrations with increased exposure periods and little evidence of adaptation was observed.

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